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Sgs1—The Maestro of Recombination

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The Sgs1 DNA helicase and its mammalian homolog BLM control crossover formation in mitotic cells. Zakharyevich et al. and De Muyt et al. now uncover a key role for Sgs1 in meiotic crossover regulation, which in turn reveals a joint molecule resolution pathway that produces the majority of crossovers in budding yeast.

Recombination between homologous chromosomes during meiosis is essential for the formation of crossover (CO) products that ensure proper chromosome segregation at the first meiotic division and impart diversity to gametes. Homologous recombination is also invoked during DNA damage repair and DNA replication restart in mitotic cells, but this occurs primarily between sister chromatids and is thus genetically silent. When mitotic recombination occurs between homolog chromosomes or repeated sequences located on different chromosomes, there is the potential for loss of heterozygosity and the uncovering of deleterious recessive mutations or chromosome rearrangements. This potential can be realized when the recombination intermediates (Holliday junctions [HJ]) are resolved as CO products. Therefore, it is important for CO formation to be regulated. The RecQ helicase Sgs1 of budding yeast, and its human counterpart BLM, have been known for some time to suppress CO formation in mitotic cells; thus, one might have expected Sgs1 not to function in meiosis, for which COs are essential. The important role of Sgs1 in meiosis had been missed until now because the null mutant shows close to normal levels of CO and NCO (noncrossover) products (Rockmill et al., 2003). Surprisingly, Zakharyevich et al., (2012 [this issue of *Cell*]) and De Muyt et al. (2012) show that Sgs1 is the master controller of meiotic recombination and determines whether a recombination intermediate becomes an early noncrossover product or is directed toward a pathway that ultimately ends up as a CO.

Strand invasion intermediates are generated by Rad51 (and/or Dmc1 in meiotic cells) catalyzed pairing between a 3′ single-stranded DNA overhang resulting from end resection of a double-strand break (DSB) and a homologous duplex. After priming DNA synthesis, the invading strand can be displaced and annealed to the other DSB end to yield a NCO (Figure 1). Alternatively, the strand invasion intermediate is stabilized, and after capturing the other DSB end, gaps are filled and ligated to form a double Holliday junction intermediate (dHJ), which is subsequently dissolved or resolved to form a NCO or CO product. The outcome of homologous recombination—NCO or CO products—differs between mitotic and meiotic cells, suggesting that different cell types have active mechanisms to regulate COs. There would seem to be at least three levels at which CO formation could be regulated. The first would be to undo recombination intermediates such that they cannot progress to the HJ stage; the second would be to resolve HJs in a NCO mode when COs should not occur; and the third would be to regulate the actual endonucleases that cleave HJs (known as resolvases). The first level is achieved by DNA helicases to unwind a strand invasion displacement loop (D loop) intermediate and force all recombination to be NCO. The second level invokes a DNA helicase acting in conjunction with a topoisomerase to dissolve a dHJ intermediate through branch migration and decatenation to form a NCO (Figure 1). The prime player in this level is Sgs1 in yeast and BLM in mammalian cells (Wu and Hickson, 2003). Indeed, loss of Sgs1 or BLM activity results in increased mitotic COs (Ellis et al., 1995; Ira et al., 2003). Early recombination models assumed that HJs could be removed by unbiased resolution to form CO or NCO products. However, molecular analysis of meiotic recombination intermediates in yeast revealed that dHJs are processed to form only COs in a manner that is dependent on Cdc5, a polo-like kinase (Allers and Lichten, 2001). In contrast, NCOs appear earlier, independent of Cdc5 activation and dHJ resolution. In addition to Cdc5 regulation, a group of meiosis-specific proteins referred to as the ZMM family is required to stabilize joint molecules (JMs) and for most meiotic COs. These results indicate that meiotic cells have distinct mechanisms in place to mature strand invasion intermediates to dHJs and to then resolve them only as COs.

Given the importance of resolvases in HJ cleavage and CO formation, the central question that De Muyt et al. and Zakharyevich et al. set out to address is: which of the known nuclease(s) is responsible for dHJ resolution in meiosis (De Muyt et al., 2012; Zakharyevich et al., 2012)? Biochemical studies had identified three structure-selective endonucleases with the potential to cleave HJs: Mus81-Mms4 (MUS81-EME1 in human), Yen1 (GEN1), and Slx1-Slx4 (BTBD12/SLX4). Each of these activities was systematically eliminated, and the contribution to resolution of JMs and generation of COs and NCOs were determined in Sgs1^{+} or Sgs1^{-} cells undergoing meiosis.

In the absence of Sgs1, JMs accumulate and are often aberrant (e.g., multichromatid JMs); resolution of these JMs yields both COs and NCOs and requires Cdc5. The JMs that accumulate in the *sgs1* mutant are primarily resolved by Mus81-Mms4, consistent with the recent finding that Mms4 is activated by Cdc5

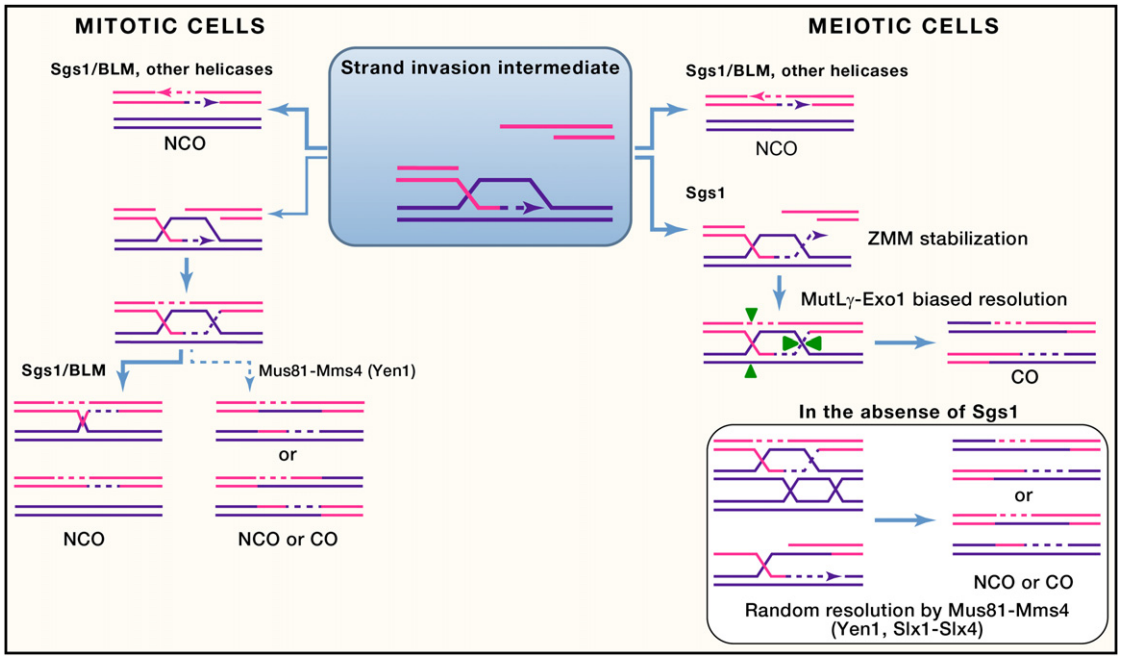

Figure 1. Sgs1 in Its Anticrossover and Procrossover Roles

In mitotic cells, recombination-based repair of a double-strand break results in a strand invasion intermediate, which can be unwound by Sgs1/BLM or other helicases to prevent crossovers (COs). If the strand invasion intermediate survives and proceeds to a dHJ molecule, this is dissolved by Sgs1/BLM in association with topoisomerase 3 and additional factors to also give noncrossover (NCO) products. Those dHJ molecules that escape Sgs1 action are resolved by Mus81-Mms4 or Yen1 in a random mode. In meiosis, Sgs1 has three roles. First, it reverses the strand invasion intermediates to form NCOs. Second, in a pro-CO mode, it prevents multichromatid strand invasion molecules from forming and promotes association of some legitimate strand invasion intermediates with the ZMM complex. Those molecules are eventually resolved in a CO mode only, mainly by MutLγ-Exo1. The molecules observed in the absence of Sgs1 are the target of Mus81-Mms4, Yen1, and Slx1-Slx4, which resolve in a random mode to give both CO and NCO outcomes.

(Matos et al., 2011). Yen1 functions as a cryptic resolvase and is only called into play in the absence of Mus81-Mms4. The contribution of Slx1-Slx4 to resolution is minor and is only revealed in the absence of Mus81-Mms4 and Yen1. Surprisingly, when Sgs1 is active, the known structure-selective nucleases generate only about 20% of the COs, and this is mainly due to Mus81-Mms4 activity. Having thus eliminated the known activities defined by vitro HJ resolution assays, Zakharyevich et al. turned to MutLγ (Mlh1-Mlh3), as earlier studies had shown that Mlh1 localized to chiasmata and was required for COs in mouse (Baker et al., 1996). Intriguingly, biochemical studies had revealed an endonuclease activity associated with the human MutL homolog, Pms2, and the conserved endonuclease motif of Mlh3 was essential for meiotic crossovers (Nishant et al., 2008), suggesting that MutLγ could be the major meiotic resolvase. However, MutLγ has no demonstrated resolvase activity in vitro. Using the JM resolution assay, Zakharyevich et al. found that most JM resolution to form COs required MutLγ together with the Exo1 nuclease.

Now, with evidence that MutLγ was the fourth resolvase, Zakharevich et al. could show that, when all four resolvases were eliminated, the level of COs was greatly reduced, but NCOs still formed in an Sgs1-dependent manner. Thus, Sgs1 is the main activity for NCO products in meiosis, but these seem to form by strand displacement rather than by dHJ dissolution. The massive accumulation of the JMs and paucity of COs in the *mms4 slx4 yen1 sgs1* quadruple mutant indicates an additional role for Sgs1 in promoting ZMM-MutLγ-Exo1-mediated resolution.

Together, these findings show that Sgs1 plays a central role in coordinating the orderly progression through the meiotic recombination pathway and strongly suggest that MutLγ is the meiotic resolvase. Sgs1 displaces the invading strand of JMs to form NCOs and prevents the accumulation of multichromatid JMs, and also shepherds a subset of strand invasion intermediates to the ZMM proteins for stabilization and maturation to dHJs. At this step, the dHJs must be protected from the dissolution activity of Sgs1. A number of questions arise from these studies. In mammalian cells, does BLM or another RecQ helicase regulate meiotic COs (mammals have five RecQs)? Does MutLγ exhibit HJ resolvase activity in vitro? How does biased resolution of dHJs occur? Is the role of Sgs1 in promoting meiotic resolution simply to prevent formation of complex intermediates or does it play a direct role with MutLγ and Exo1? Further work will be required to resolve these issues.

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Growing Cells Push Back under Pressure

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In both plants and animals, the interplay between mechanical force generation and mechanical sensing plays a stabilizing role in many developmental processes. Uyttewaal et al. now demonstrate that cells in the *Arabidopsis* shoot apical meristem respond to local mechanical stresses by reorienting their growth, thereby guiding morphogenesis. Notably, the mechanism underlying such guidance is amplification—not suppression—of growth-rate heterogeneity.

Genetic methods have been extraordinarily powerful for the functional dissection of developmental processes, but they have a limited capacity to elucidate the role of physical forces in morphogenesis. As a growing tissue is necessarily constrained by its geometric form and emergent mechanics, developmental molecular-genetic programs are inextricably linked to biophysical feedback. Although the instructive role of mechanical stress in development is beginning to be understood at the cell and tissue levels, feedbacks between these scales as a function of time are formidably complex—especially in proliferating cellular systems—and constitute an important challenge for the field (Aigouy et al., 2010; Desprat et al., 2008; Hamant et al., 2008; Rauzi et al., 2008; Savin et al., 2011). Uyttewaal et al. (2012) now take an important step toward understanding the interplay between mechanical signaling and active growth control in the *Arabidopsis* shoot apical meristem (SAM). They identify a local positive feedback mechanism that increases differences in the direction and rate of cell growth across the tissue in response to mechanical stress. This mechanism is required to maintain normal meristem shape and thus links active remodeling of the cytoskeleton to robust organ morphogenesis.

In the *Arabidopsis* SAM, the authors have previously demonstrated that the orientations of cortical microtubule (CMT) arrays correlate with the principal direction of stress in this tissue (Figure 1A) (Hamant et al., 2008). In the present study, the authors adopt a nematic tensor-based approach that quantitatively describes both the locally predominant CMT orientation and CMT orientation variability. A similar method was used recently to study planar polarity protein distributions in *Drosophila* (Aigouy et al., 2010). The authors first confirm that CMT arrays reorient with slower dynamics in the tissue of a microtubule-severing mutant, *katanin*. They next show that in *katanin* mutants, neighboring cells tend to grow more frequently in the same direction, and that the characteristic dome-like shape of the shoot tip inverts (Figures 1B, 1C, 1B′, and 1C′). By combining physical measurement of tissue mechanics, mechanical compression, laser ablation, pharmacological manipulation of intrinsic tissue stresses, imaging of CMT arrays, and mathematical modeling, the authors then test the central hypothesis that the *katanin* mutant's morphological defects are the result of its failure to react sufficiently strongly to mechanical stress, rather than being an artifact of emergent mechanical differences in the mutant tissue.

Based on these approaches, the authors reach a number of intriguing conclusions. First, the laser-ablation and compression tests strongly suggest that *katanin* mutants are deficient in their ability to react to stress. Second, using a simple vertex model of the SAM in which cells locally reorient their growth to avoid elongating in the direction of maximal stress, the authors predict—and then experimentally verify—that in the wild-type tissue, active stress-responsive CMT arrays can actually increase the heterogeneity of growth rates if the mechanical feedback is sufficiently strong. This is a completely different result from that